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Cytochrome P450 1A induction in gudgeon (*Gobio gobio*) : Laboratory and Field Studies

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The induction of cytochrome P450 1A was studied in gudgeon (*Gobio gobio*), a common European cyprinid, using both farm-raised and field-caught fish. The effects of sex, reproductive status and past exposure to xenobiotics were assessed. When exposed to beta-naphthoflavone (bNF), reared gudgeon showed a dose-dependent increase of EROD activity with a plateau observed at doses from 20 mg kg^{-1} (females) and 5 mg kg^{-1} (males). The sexual difference in EROD activity was related to the gonadosomatic index (GSI) of the female whatever the level of induction. Dose and sex effects were confirmed by the immunodetection of CYP1A protein. More than 1 month was necessary for EROD activity to decrease to baseline levels. A second bNF injection after 32 days gave similar levels of induction, suggesting that EROD induction by bNF was not impaired by a pre-treatment. Wild fish were brought from two sites in the Rhône river basin: a low contaminated site (Ain) and a highly contaminated site (Rhône). Wild gudgeon were highly induced by bNF in laboratory conditions, except males from the Rhône site which exhibited EROD levels as high as the EROD plateau found in laboratory conditions. A 2-month depuration period in clean water was necessary for EROD activity in wild gudgeon to decrease to baseline levels. These results provide better knowledge of the main factors of modulation of the induction in gudgeon as well as on the influence of the history of exposure to inducers.

Keywords: EROD, fish, *Gobio gobio*, biomonitoring.

Introduction

Among biochemical biomarkers, the measurement of fish EROD activity (ethoxyresorufin *O* deethylase activity, a cytochrome P450 1A dependent monooxygenase) is one of the best documented responses to contaminants such as dioxins, polycyclic aromatic hydrocarbons (PAHs), polychlorobiphenyls (PCBs). Yet, the degree of induction of EROD activity may vary depending on species, sexual maturity, feeding behaviour, inhibitors, etc. (Goksøyr and Förlin 1992). To improve our ability to accurately interpret data from field studies, it is essential to quantify the factors of EROD variability in both field and laboratory conditions as well as to conduct controlled laboratory experiments on sentinel fish species.

Many assumptions made from field observations can only be studied in laboratory experiments, such as studies on the species sensitivity to induction (Förlin and Celander 1993), time-course of induction over extended periods (Van der Weiden *et al.* 1993, Huuskonen *et al.* 1995, Eggens *et al.* 1996), natural factors of variability (Förlin *et al.* 1984), and inducibility and reversibility of EROD levels of fish continuously exposed to inducers (Wirgin *et al.* 1992, Celander and Förlin

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1995, Förlin and Celander 1995, Prince and Cooper 1995). However, a few studies, using caging techniques, have measured EROD activity in the same fish species both in field and in laboratory conditions (Jimenez *et al.* 1988, Oïkari and Lindström-Seppä 1990, Jedamski-Grymlas *et al.* 1994, Fenet *et al.* 1996).

In western European continental waters, gudgeon (*Gobio gobio*), a common and non-migratory cyprinid, is an appropriate sentinel species. We studied gudgeon EROD induction in fish from the field (Flammarion and Garric 1997, Flammarion 1998). In addition, the small size of gudgeon enables easy holding in the laboratory, and gudgeon can be obtained both from the field and from fish farms.

The objective of the study was to describe the induction of cytochrome P450 1A in gudgeon from the field and from the laboratory, taking into account three main factors: dose of inducing compound, sexual maturity, and history of exposure to pollutants. In the laboratory, this was done by evaluating the effects on EROD variability by reproductive status (quantified as gonadosomatic index, GSI) and inducing pollutants (modelled as beta-naphthoflavone, bNF). A multiple bNF injection was also carried out to study the EROD response in the case of successive exposures. In addition, we compared the induction of cytochrome P450 1A in fish from two distinct sites, and we examined the EROD inducibility and reversibility with wild gudgeon brought to the laboratory.

Materials and methods

Chemicals

7-Ethoxyresorufine, reduced β-nicotinamide adenosine-diphosphate (NADPH), beta-naphthoflavone (bNF), and phenylmethylsulphonylfluoride (PMSF) were purchased from Sigma Chemicals Co. (St Louis, MI, USA). Aroclor 1254 was obtained from Cluzeau (Sainte Foy la Grande, France). Monoclonal antibodies mouse anti-cod CYP1A IgG NP-7 were obtained from Biosense Laboratories AS (Bergen, Norway). All other chemicals were of the highest available commercial grade.

Sampling of wild fish from two river locations

The sampling sites were located in the Rhône watershed (south-east of France): one low polluted area (Blyes in the Ain river) and one highly polluted area (Sablons-Serrières in the Rhône river). Metals and organics are the major contaminants in the Rhône site which is located downstream from a large industrial area, whereas the Ain site is a reference area (Flammarion and Garric 1997, Flammarion *et al.* in press).

Gudgeon (*Gobio gobio*) were sampled in spring (May–June : cyprinids spawning period) and in autumn (September–October). The maximum time interval between samplings of the two sites was 1 month. After being captured by electrofishing, fish were immediately sacrificed, weighed, measured, and dissected.

Experimental fish holding

Gudgeon (107 ±0·6 cm ; 12 ±3 g) were obtained from a local fish farm in central France (C. Giraud, Fournols, France). Fish were held for at least 2 weeks before injection in dark tanks supplied with a water flow rate of 1·2 l kg^{-1} min^{-1} (temperature = 17 ±1°C; conductivity = 490 ±10 µS cm^{-1}; pH = 7·2 ±0·2; dissolved oxygen = 8·0 ±0·5 mg l^{-1}). Fish were fed Trouvit (Trouw, Fontaine-les-Vervins, France) at a rate of 1 % body weight a day. Feeding was stopped 24 h before both injection and sacrifice. Fish were maintained on an 8:16-h dark:light photoperiod. All reared gudgeon were spawning fish (experiments 1 and 2).

Experiment 1 : cytochrome P4501A induction by bNF in reared gudgeon—dose response and sex differences

Cytochrome P450 1A induction was obtained by intraperitoneal injection (i.p.) with bNF in peanut oil. Each dose (0 ; 0·5 ; 1 ; 2 ; 5 ; 10 ; 20 ; 50 ; 100 mg bNF kg^{-1}) was administered once as 100 µL per 10 g to female and male gudgeon in late spring. Exposure was 4 days long.

Experiment 2 : reared gudgeon—time dependence of induction and repeated injection

Gudgeon were sampled in late spring and were treated with 50 mg bNF kg^{-1} and dissected 4, 8, 16 and 32 days later. Then, 32-day pre-treated fish were injected with 1 or 50 mg bNF kg^{-1} and dissected 4 days later to test the influence of a repeated injection on the induction of EROD activity.

Experiments 3 and 4 : wild gudgeon—inducibility by bNF and reversibility of induction

For two studies, wild fish were gently brought to the laboratory and dissected either 3 days after a 50 mg bNF kg^{-1} injection, or 2 months after a depuration study in clean laboratory water. Tank water was maintained at the same temperature and conductivity as in the field site. With the depurated fish, chemical analyses in muscle were performed so as to compare the potential parallelism, if any, between the decrease of EROD activity induction and the decrease of bioaccumulated pollutants.

EROD assay

After fish were sacrificed, liver and gonads were removed. Liver was rinsed in 150 mM KCl, homogenized in 100 mM phosphate buffer, pH 7·8 with 20 % glycerol and PMSF, poured into 1·8 ml cryotubes. Liver homogenates were frozen in liquid nitrogen and kept for several days at –80°C before enzymatic assay. Gonadosomatic index (GSI) was calculated and expressed as the ratio of gonads weight to total body weight. This was done only in the laboratory with experimental fish because of the low precision of the field balance.

Liver homogenates were thawed at 4 °C and centrifuged at 9000 *g*. The supernatant (S9) was used for enzymatic assay performed at room temperature (20 ± 1 °C) by a fluorimetric procedure on a 96-well microplate (Flammarion and Garric 1997). Enzymatic activities were expressed in terms of S9 protein concentrations measured by the method of Lowry *et al.* (1951).

Preparation of microsomal fractions

Just after EROD dosage, S9 supernatants were pooled and centrifuged at 105 000 *g* for 1 h at 4 °C. The microsomal pellets were resuspended in homogenization buffer (100 mM phosphate, pH 7·8, 1 mM EDTA, 1mM DTT, 20 %glycerol). Microsomal proteins were determined according to Lowry *et al.* (1951).

Western blotting

SDS–PAGE gels (10 % polyacrylamide) were run according to the method of Laemmli (1970). Microsomal proteins (20 µg loadings) were transferred to nitrocellulose membrane and immunodetected using enhanced chemiluminescence (ECL, Amersham Life Sciences, Little Chalfont, UK), which is 10-fold more sensitive than colorimetric methods (Kloepper-Sams and Benton 1995). The immunodetection procedure used a mouse anti-cod CYP1A monoclonal antibody (Biosense Laboratories, Bergen, Norway) at a 1/200 dilution and an anti-mouse horseradish peroxidase (HRP)-linked secondary antibody (BioRad, Hercules, CA, USA) at a 1/1000 dilution. The bound HRP catalyses an enhanced chemiluminescent reaction producing light captured on film (1 min exposure, Kodak Biomax film). Image recording and processing were performed by Visilog 4.1 (Noesis, France).

Chemical analysis of fish muscle

The chemical determination of bioaccumulated pollutants in gudgeon was performed with gudgeon from the Rhône site and brought to the laboratory for the depuration study. This is a multiresidue procedure suitable for the determination of 132 molecules (EC 76/464) including PCBs, organochlorine pesticides and PAHs. In this study, only PCBs were detected in the fish muscle. Muscles obtained at the same sampling date were pooled.

PCB analyses were performed on a gas-chromatograph (GC) with electron-capture detection (ECD). A 10-g portion of homogenized muscle was extracted twice (neutral, acidic) with 50 ml dichloromethane/acetone (1 : 1) using an Accelerated Solvent Extraction (ASE 200 Dionex) for 5 min at 100 °C. The organic extracts were combined prior to 2-h freezing and final concentration to 10 ml in ethyl acetate. GC/ECD was performed with a Varian Star 3400 apparatus using a Varian 8200 injector (1 µl injection). The columns used were DB5 (J&W Scientific) and SGE capillary columns. Nitrogen was the carrier gas. The injector was heated up to 300 °C at 150 °C min^{-1} and held for 34 min.

PCB results were given as the sum of 12 congeners (PCB 28, 44, 52, 101, 105, 118, 138, 153, 170, 180, 194, 209) in µg g^{-1} lipid weight.

Data analysis

STATISTICA software (StatSoft Inc., Tulsa, OK, US) was used for all statistical analyses (ANOVA, regression, Student's *t*-test). EROD and GSI data were automatically log-transformed to

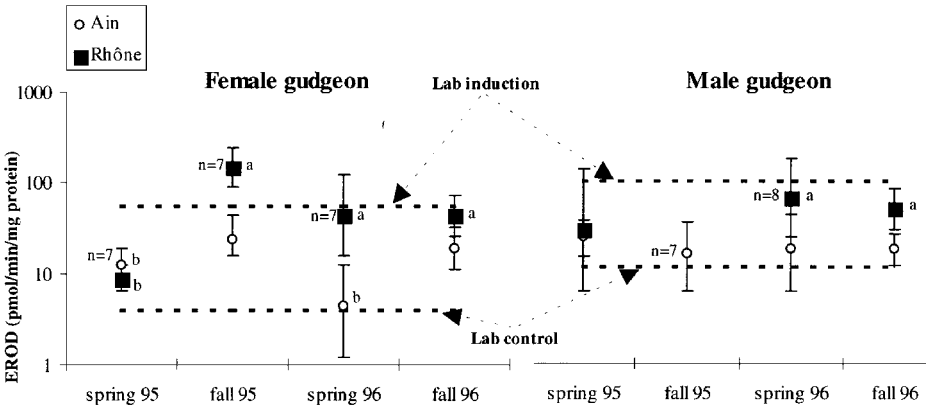

Figure 1. Comparison of field EROD levels (pmol min^{-1} mg^{-1} protein) with control and 4-day 50 mg bNF kg^{-1}-induced laboratory gudgeon. EROD activities in fish from Ain (O) and Rhône (■) are given as geometric means with 95% confidence intervals. Dashed lines represent the reared-fish EROD levels (do not suggest that laboratory studies were performed continuously over the 1995–96 period). Unfortunately, no males could be sampled from the Rhône in autumn 1995. Samplings were n=10 unless otherwise noted in the figure. a: significantly different from Ain; b: significantly different from males.

conform to the normality test (χ^2 test for normality) and to the homogeneity of variance (Levene's test). The 95 % confidence intervals (95 % CI) were calculated on the log-transformed data using the critical values of Student's t-distributions.

Results

CYP 1A induction in wild and reared gudgeon

A 2-year study of EROD levels in gudgeon from Ain and Rhône showed that EROD activities in the Rhône site were 5–10 times higher than in the Ain station (figure 1). Sexual differences in EROD activities were greater in spring, the spawning period. Temporal differences in the Rhône results might be due to the temporal variability of pollution in the area downstream of a large industrial zone (e.g. higher inducing contamination in spring 1996 than in spring 1995).

In the laboratory, gudgeon baseline EROD activities did not vary significantly over the course of the experiments (6 months) for both males and females, with geometric means (and 95 % CI) of baseline EROD activities for females and males of 4 (2–7) (N = 37) and 12 (7–17) (N = 44) pmol min^{-1} mg^{-1} protein respectively. In addition, EROD induction by 50 mg bNF kg^{-1} (sampled 4 days after treatment : experiments 1 and 2) showed reproducibility for both females and males (the experiment was repeated twice). The bNF-treatment led to a 10-fold induction of EROD activity in gudgeon for females and males : 54 (24–120) (18) and 109 (90–134) (19) pmol min^{-1} mg^{-1} protein respectively (figure 1).

Those experimental results show the reproducibility of the experiments with gudgeon in the laboratory. In addition, the control and induced EROD activities observed in the laboratory were quite similar to control and induced field EROD levels (figure 1).

The induction of cytochrome P450 1A in gudgeon from the Rhône site was confirmed by the immunodetection of CYP1A protein (figure 2). The anti-cod

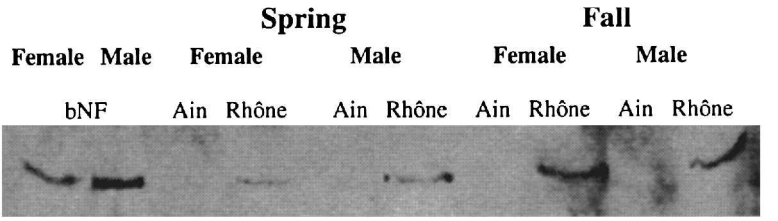

Figure 2. Immunoblotting of gudgeon hepatic microsomes for cytochrome P4501A using anti-cod Mab. Reared and wild fish were sampled in 1996. Loadings of 20 µg microsomal proteins per lane of the gel.

CYP1A Mab recognized a protein band of 58 kDa with microsomes from both bNF-treated and field-induced gudgeon.

Experiment 1 : cytochrome P4501A induction by bNF in reared gudgeon—dose response and sex differences

Maximum EROD activities were observed for males at doses of 5–100 mg kg^{-1} and for females at doses of 20–100 mg kg^{-1} (figure 3). No sexual difference in EROD activity was detected at 50 and 100 mg kg^{-1}, suggesting that female EROD activity plateau was as high as that for males (i.e. about 100 pmol min^{-1} mg^{-1} protein). The 0·5 mg bNF kg^{-1} i.p. injection was enough to significantly induce EROD activities of both females ($p = 0{\cdot}04$) and males ($p < 0{\cdot}01$) whereas CYP1A protein was not detected at this dose using Western blotting (figure 4).

A two-way ANOVA was performed to test for differences due to dose and sex. At doses <20 mg kg^{-1}, dose and sex effects were highly significant ($p < 0{\cdot}001$) while the interaction was not significant ($p = 0{\cdot}20$). This confirmed that the EROD activities of males were higher than those of females, whatever the dose < 20 mg kg^{-1}.

In addition, the female within group variability was higher than that for males (figure 3). The EROD within group variability was quantified with the SD of log(1+EROD) distribution. No dose effect on such dispersion was observed, whereas a strong sex effect was detected ($p < 0{\cdot}01$) : SD(females) = $1{\cdot}07 \pm 0{\cdot}29$ and SD(males) = $0{\cdot}45 \pm 0{\cdot}17$.

The higher variability observed for females may be due to the degree of sexual maturity. Within each bNF treatment, we observed a significant relationship between EROD and GSI. That was found only with females, and both intercept and slope values of the linear regression analyses were dose dependent (Table 1). Then, we looked for a more general model such as log(1 + EROD) as a function of GSI : if « ratio » is the log(1 + EROD) level for a female normalized with the corresponding mean for males with the same bNF treatment, then:

$$\text{ratio} = \frac{\log(1 + \text{EROD})(\text{female})}{\text{mean}(\log(1 + \text{EROD}))(\text{males})} \quad 1{\cdot}3 - 0{\cdot}5\log(1 + \text{GSI}) (R^2 = 0{\cdot}5;\ p < 0{\cdot}05)$$

Experiment 2 : reared gudgeon—time dependence of induction and repeated injection

After a 50 mg bNF kg^{-1} injection, EROD induction was constant for females and for males for 4, 8, and 16 days (figure 5). However, after 32 days, female EROD

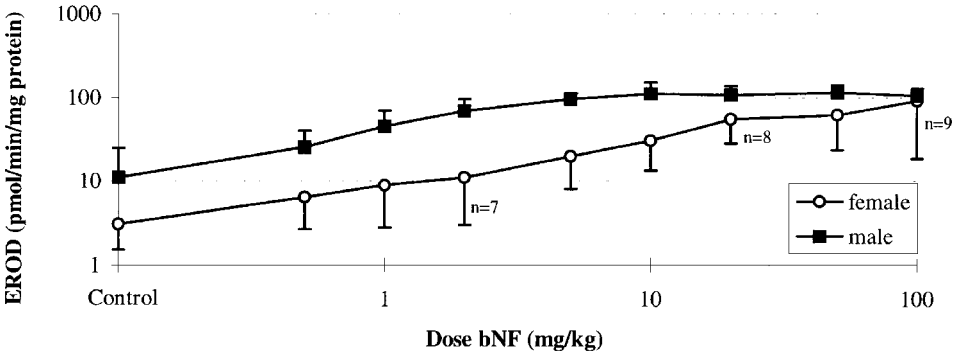

Figure 3. Gudgeon EROD activities (pmol min^{-1} mg^{-1} protein) 96 h after i.p. injection of bNF. Values are given as geometric means with 95 % confidence intervals. Samplings were $n = 10$ unless otherwise noted in the figure.

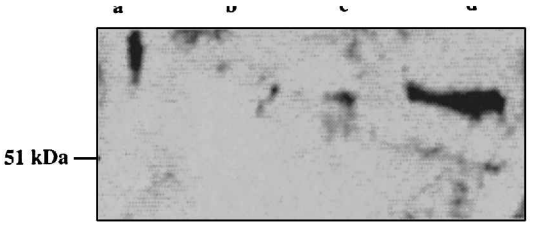

Figure 4. Male gudgeon CYP1A immunodetection 96 h after i.p. injection of bNF. Lanes a, b, c, and d : 0 ; 0·5 ; 5 and 50 mg bNF kg^{-1} respectively.

activities had significantly decreased and were not different from control ($p = 0{\cdot}5$). Male EROD activities had also significantly decreased, but they were still higher than control ($p < 0{\cdot}05$) (table 1). Then, we tested the inducibility of EROD activity in 32-day bNF pre-treated gudgeon with an additional injection of 1 or 50 mg bNF kg^{-1}. These doses were chosen because the first one evaluates the EROD sensitivity while the second dose evaluates the maximum of induction (considering the dose–response curve in figure 3). EROD activities after an additional injection were consistent with those of the dose–response curve (figure 3).

Experiment 3 : wild gudgeon—inducibility by bNF

An additional induction was performed on fish from both sites (Ain and Rhône) to test the responsiveness of EROD induction. A 3-day bNF experiment at a 50 mg kg^{-1} dosing significantly induced ($p < 0{\cdot}01$) EROD activities of all fish groups from Ain and Rhône, except males from Rhône (figure 6) whose EROD activities were as high as the EROD plateau (see figure 3). In addition, identical EROD induction was obtained with gudgeon from both Ain and Rhône. This suggests that a maximum induction was obtained.

Experiment 4 : wild gudgeon—reversibility of induction

Gudgeon from the Rhône site were placed into laboratory water to observe the potential EROD decrease over several months. No sexual EROD difference was

Table 1. EROD activities of control and bNF-previously treated gudgeon after receiving a 4 day-bNF treatment. Values are given as geometric means with 95 % confidence intervals and number of fish in parentheses.

	EROD activity (pmol min^{-1} mg^{-1} protein)					
	Female			Male		
	Control	1 mg kg^{-1}	50 mg kg^{-1}	Control	1 mg kg^{-1}	50 mg kg^{-1}
Non pre-treated fish	4 (2–7) (37)[b]	9 (4–16) (10)	54 (24–120) (18)[b]	12 (7–17) (44)[b]	44 (29–72) (10)	109 (90–134) (19)[b]
Pre-treated fish[a]	6 (3–12) (10)	14 (6–35) (10)	70 (44–108) (10)	29* (15–54) (10)	63 (29–120) (8)	98 (59–163) (8)

[a] Gudgeon was first injected with 50mg bNF kg^{-1} 32 days prior to the second injection.
[b] Pooled results (see first section of the results).
* EROD activity in pre-treated fish significantly different from control non pre-treated fish ($p<0{\cdot}05$).

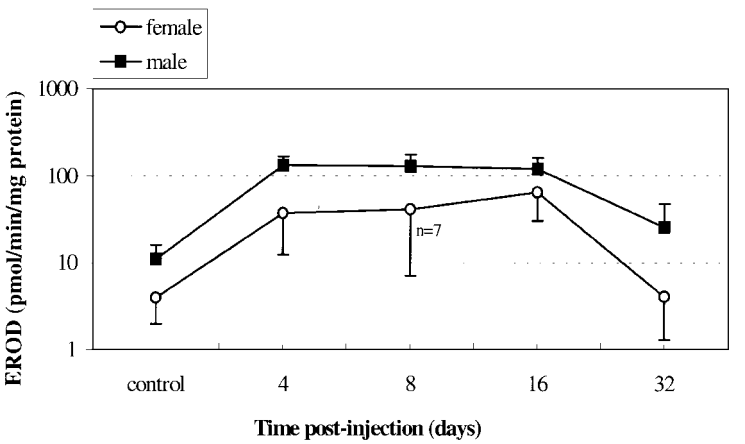

Figure 5. Time course of EROD activities (pmol min^{-1} mg^{-1} protein) in female and male gudgeon injected with 50 mg bNF kg^{-1}. Values are given as geometric means with 95 % confidence intervals. Samplings were $n = 10$ unless otherwise noted in the figure.

observed because fish were sampled in autumn, so only pooled results of adult gudgeon are shown in figure 7. The EROD decrease was significant (about –60 %) after 1 month and EROD activities were as low as levels of Ain (in autumn) after 2 months. Only PCBs were detected in gudgeon muscle from the Rhône site. PCBs levels were also found to decrease over the duration of the experiment (figure 7).

Then, we compared the inducibility (with 50 mg kg^{-1} of Aroclor 1254 and dissection 4 days after the injection) in reared gudgeon and in depurated wild gudgeon (table 2). Induced EROD activities were equivalent, suggesting the integrity of inducibility in wild fish from the Rhône site.

Discussion

This study has improved our knowledge about the variability of cytochrome P450 1A induction in gudgeon under both laboratory and field conditions, taking

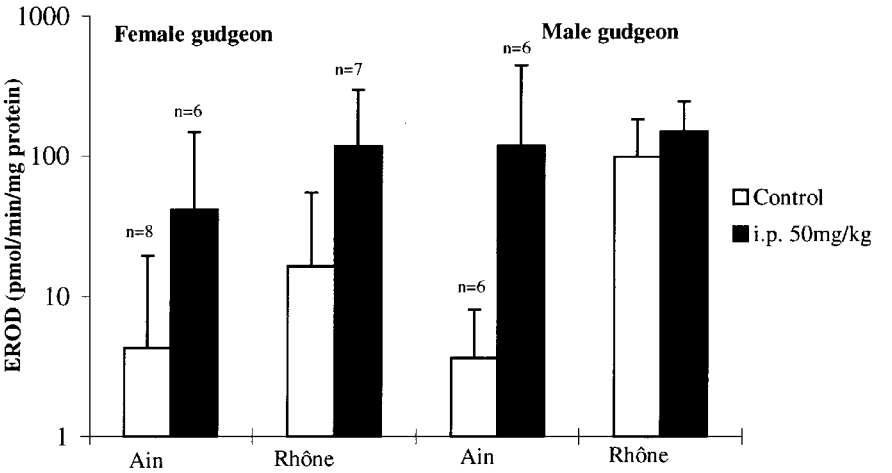

Figure 6. Effect of a 50 mg bNF kg^{-1} treatment on EROD activities (pmol min^{-1} mg^{-1} protein) of gudgeon from two different sites in spring 1996. Fish were sacrificed 3 days after treatment. Values are given as geometric means with 95 % confidence intervals. Fish had also been dissected the day of bNF-treatment to test the laboratory influence during the 3 days (no influence was observed). Samplings were $n = 10$ unless otherwise noted in the figure.

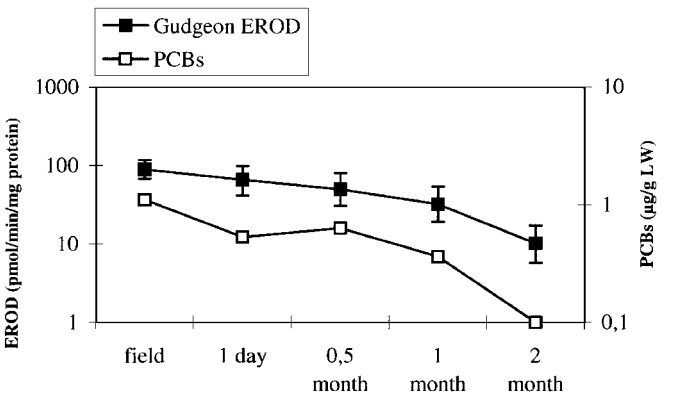

Figure 7. Effect of a clean water depuration on EROD activity (pmol min^{-1} mg^{-1} protein) in gudgeon from the Rhône site sampled in autumn 1996. EROD values are given as geometric means with 95 % confidence intervals. Since no sex differences were observed for EROD activities, pooled results ($n = 20$) were displayed. PCB data points are results from chemical analyses on pooled muscle, so no error bars were calculated.

exposure, sexual maturity and history into account. This study represents the first analysis on the EROD response in gudgeon from both the field and a fish farm.

Gudgeon proved to be a suitable species for laboratory and field studies. We have previously also used chub (*Leuciscus cephalus*) in field studies to quantify the EROD induction downstream of a chemical plant (Vindimian *et al.* 1991) and a wastewater treatment plant (Kosmala *et al.* in press). In addition, we quantified both chub sexual difference in EROD activity as well as the relationship between EROD and the bioaccumulated organochlorindes (Flammarion 1998). However, because of its large size, it was not possible to hold adult chub under laboratory conditions which would allow further quantification of the relationship

Table 2. Effect of a 50 mg kg^{-1} i.p. injection of Aroclor 1254 Aroclor 1254 on PCBs levels in muscle and on EROD activity in laboratory gudgeon and in 2-month-depurated gudgeon from the Rhône site. EROD values are given as geometric means with 95 % confidence intervals and number of fish in parentheses.

Treatment	PCBs levels (μg g^{-1} LW)		Female EROD activity (pmol min^{-1} mg^{-1} protein)		Male EROD activity (pmol min^{-1} mg^{-1} protein)	
	Lab fish	Wild fish	Laboratory fish	Wild fish	Laboratory fish	Wild fish
Control	ND	0·1	5 (3–8) (10)	7 (3–18) (10)	15 (9–25) (10)	12 (5–28) (10)
Aroclor 1254	20·0	20·9	32* (13–80) (10)*	27* (13–52) (10))*	44* (21–80) (10)*	37* (17–79) (10)*

between sex and EERODinduction. But, results obtained with immature chub, brought from the same field sites, confirmed conclusions made with gudgeon as far as inducibility and reversibility are concerned (Flammarion 1997).

Our study dealt with wild fish kept in the laboratory. This is a matter of concern because of possible alterations of monooxygenase enzyme activities after a change of the fish habitat : the transfer of the fish to laboratory, although gently accomplished, may be stressful and cause a decrease in EROD activity (Huuskonen *et al.* 1995). Yet, we did not observe such a decrease when field-induced fish had been transferred to the laboratory just after electrofishing. In addition, fish from the control site responded to bNF treatment in the same range as reared gudgeon.

A dose–response curve of EROD activity in gudgeon injected with bNF was determined. The results are in agreement with earlier studies of fish treated with bNF. EROD activity was induced at 0·5 mg kg^{-1} as already observed with rainbow trout (Flammarion *et al.* 1996) and EROD activity appeared to plateau at ~10–20 mg kg^{-1}. The maximum of EROD induction was reached at doses of 10–50 mg kg^{-1} and after 3–4 days of exposure (Melancon *et al.* 1981, Kloepper-Sams and Stegeman 1989, Stegeman *et al.* 1990, Celander *et al.* 1993). Yet, CYP 1A induction may have occurred between 5 and 50 mg kg^{-1} in the male (figure 4), whereas the EROD activity is at the plateau. This could result from an inhibition of the EROD activity at high doses of bNF as already observed (Stegeman *et al.* 1990, Haasch *et al.* 1993).

Reared gudgeon were sexually mature fish which explains the difference between females and males in both control and induced EROD levels. Measurement of GSI allows quantification of gudgeon sexual maturity (Kestemont 1987), and GSI levels were found to be positively related to female oestradiol-17β with barbel (Poncin 1988) and rainbow trout (Förlin and Haux 1990). We quantified the sexual difference in EROD activity which was found to be negatively related to the GSI levels of female gudgeon just prior to spawning. This is in agreement with the inhibitory effect of oestrogens: administration of oestradiol to juvenile *Salvelinus fontinalis* and *Oncorhynchus mykiss* resulted in significant decreases in monooxygenase activities (Stegeman *et al.* 1982); co-injection of both oestradiol-17β and bNF to cyprinid *Rutilus rutilus* resulted in almost 50 % decreases for both basal and induced EROD levels (O'Hare *et al.* 1995).

By correcting female log(1+EROD) levels with the EROD–GSI formula, we confirmed that no significant difference could be observed between males and

females in log(1+EROD) levels (a two-way ANOVA, with dose and sex as factors, did not find either significant effect of both factors or interaction). In addition, we found a decrease in the variance (38 % in place of 51 %; males : 21 %) that could not be explained by the ANOVA grouping variables (sex, dose). Such unexplained variability was already observed to be high (Collier *et al.* 1995, Flammarion *et al.* in press). The global relationships observed with bNF-treated female gudgeon between EROD and GSI are quite similar to those found with wild female gudgeon brought into the laboratory (ratio = 1·3 –0·35 log(1+GSI) (r^2 = 0·5 ; F = 20 ; N = 19)) (data not shown) and with adult female chub (ratio = 1·0 – 0·25 log(1+GSI) (r^2 = 0·2; F = 15 ; N = 56)) (Flammarion *et al.* in press). This might be of practical interest when sampling adult fish for EROD determination : only 10 adults could be analysed instead of 10 females and 10 males. In this case, a more precise field balance (accuracy < 1g ; sheltered from wind, stability checked) should be used because of the small size of gudgeon gonads. Of less practical interest but more related to the inhibition mechanism, the measurement of oestradiol levels could be performed and a similar formula could then be derived.

We observed a significant decrease of EROD activity only 1 month after an injection with 50 mg kg^{-1}. A period of 16 days was not sufficient to observe such a decrease, whereas O'Hare *et al.* (1995) observed a decrease after 6 days with *Rutilus rutilus* treated with 100 mg bNF kg^{-1}, and Kloepper-Sams and Stegeman (1989) after 12 days with *Fundulus heteroclitus* injected with 50 mg bNF kg^{-1}. The conflicting results observed in the literature concerning time dependence of EROD induction are likely to be due to the dose and kind of the inducer as well as to the fish species. Some authors reported sustained activity only with the highest concentrations while a decrease could be observed with the lowest concentrations (Muir *et al.* 1990). It was also observed that maximum EROD levels were attained rapidly and that EROD activity slowly declines or remains constant over months with TCDD (Van der Weiden *et al.* 1993) or PCB (Palace *et al.* 1996). Such discrepancies in the temporal pattern of induction may be attributed to the kinetics of metabolization of the inducer : dioxins are more slowly metabolized than PAHs and persist at active inducing levels (Celander and Förlin 1995).

In the present study, we observed that treatment of gudgeon with bNF did not alter their responsiveness towards a second injection of bNF 32 days later. In contrast, Celander and Förlin (1995) observed that a pre-treatment with PCB led to a decrease in response when fish were treated with PCB again, and a non induction when fish were furthermore injected with 3-MC. According to these authors, a possible explanation could be that the Ah receptor was being either converted or degraded. Thus, in the Rhône River, highly contaminated with PCBs and HAPs (Flammarion *et al.* in press), the prior exposure of fish could have affected their inducibility, as already observed with a prior exposure to PCB (Wirgin *et al.* 1992, Celander and Förlin 1995). Yet, our results suggest that inducibilities by bNF (immediately on arrival at the laboratory) and by Aroclor 1254 (after a 2-month depuration period) were not impaired. In contrast, Prince and Cooper (1995) observed that *Fundulus heteroclitus* populations from a chemically impacted site (with elevated EROD activities) could not be induced by TCDD, while non-impacted fish showed a great increase. This may suggest that the impacted fish were resistant to the ability of TCDD to induce EROD activity because of an alteration in the Ah receptor complex: a 90-day clean water depuration on chemically impacted fish did not decrease EROD activities and did not change their

non-inducibility. We did not observe such a phenomenon, which might indicate that the field induction in fish from Rhône is caused by more rapidly metabolized compounds. Besides, the decrease observed in fish brought into clean water suggests that these fish did not suffer from a genetic alteration, acquired over successive generations, due to the influence of heavy environmental pressure.

In conclusion, this work examined the EROD response in gudgeon both in the field and the laboratory. The quantification of the induction as a function of dose, sex and time in the laboratory provided the ranges of the induction with lower and upper limits. CYP1A protein was immunodetected by MAb anti-cod both in experimental and wild fish. In addition, taking the time-effect into consideration, studies of sensitivity and reversibility with fish from the laboratory or from the field provided better knowledge for further interpretation of EROD biomonitoring results. As such, gudgeon appears to be an appropriate fish species for biological monitoring work in the aquatic environment.

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